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Short Communication

Cross-sectional survey for *Toxoplasma gondii* infection in humans in Fernando de Noronha island, Brazil

Estudo transversal da infecção por *Toxoplasma gondii* em humanos da Ilha de Fernando de Noronha, Brasil

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Abstract

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii,* is zoonotic disease and is one of the most important foodborne parasitic diseases globally. The prevalence in humans is highly variable, being influenced by cultural habits, socioeconomic, and environmental conditions. The objective of this study was to estimate the prevalence of *T. gondii* infection in humans on the archipelago of Fernando de Noronha, Pernambuco State, Brazil, and to identify the risk factors associated with this infection. The seroprevalence of immunoglobulin G *anti-T. gondii* antibodies was 50.4% (172/341, 95% CI: 45.2%–55.7%). Factors associated with the infection were consumption of well water or rainwater (odds ratio [OR]: 2.43, p=0.020) and consumption of game meat (OR: 1.80, p=0.026). This is the first study to provide epidemiological information of *T. gondii* infection among the residents of the Island of Fernando de Noronha, revealing a considerable antibody seroprevalence in this population. This study provides information for the adoption of prevention and control measures in island environments.

Keywords: Insular environment, Seroepidemiology, Toxoplasmosis.

Resumo

A toxoplasmose, causada pelo protozoário *Toxoplasma gondii*, é uma zoonose e uma das doenças parasitárias transmitidas por alimentos mais importantes em todo o mundo. A prevalência em humanos é altamente variável, sendo influenciada por hábitos culturais, condições socioeconômicas e ambientais. O objetivo deste estudo foi estimar a prevalência de infecção por *T. gondii* em humanos, no arquipélago de Fernando de Noronha, Pernambuco, Brasil, e identificar os fatores de risco associados a essa infecção nesse contexto insular. A soroprevalência de anticorpos IgG anti-*T. gondii* nos ilhéus foi de 50,4% (172/341, 95% CI: 45,2%–55,7%). Os fatores associados à infecção encontrados foram o consumo de água do poço ou de água da chuva (*Odds ratio* [OR]: 2,43, p=0,020) e consumo de carne de caça (OR: 1,80, p=0,026). Este é o primeiro estudo a fornecer informações epidemiológicas da infecção por *T. gondii* entre os moradores da Ilha de Fernando de Noronha, revelando uma considerável soroprevalência de anticorpos nessa população. Este estudo fornece informações para subsidiar a adoção de medidas de prevenção e controle em ambientes insulares.

Palavras-chave: Ambiente insular, Soroepidemiologia, Toxoplasmose.

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Introduction

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is an important zoonotic disease that causes severe problems in humans, including reproductive, ophthalmic (Tenter et al., 2000; Montoya & Liesenfeld, 2004), neurological changes (Dubey et al., 2012) and behavioral disorders (Flegr et al., 2014). The overall range of seroprevalence was determined to be 0.5-87.7%, with an average global seroprevalence rate of 25.7% (95% CI: 25.6-25.8%) (Molan et al., 2019), which is attributed to several factors such as dietary habits, age (Dubey et al., 2012), and sanitary conditions (Mareze et al., 2019). However, these data are mainly from studies conducted in continental regions, epidemiological data in insular environments, particularly in Brazil, are scarce.

The Fernando de Noronha Archipelago, belonging to the state of Pernambuco, comprises 21 islands and islets of volcanic origin located 350 km off the coast of the Brazilian northeast in the South Atlantic. The main island that names the Archipelago has one of the highest densities of cats described in island environments worldwide (Dias et al., 2017). Magalhães et al. (2016a, b; 2017) has previously assessed infection by this coccidian in companion and production animals raised on Fernando de Noronha Island and found high seropositivity prevalence in sheep (85.0%; 204/240), pet cats (71.26%; 248/348), feral cats (54.74%; 150/247), and especially free-range chickens (88.4%; 380/430), which may indicate a high rate of environmental contamination by oocysts of the protozoan since chickens act as sentinels (Magalhães et al., 2016a; Dubey et al., 2010). Moreover, isolates of *T. gondii* have been obtained from animals from the island and molecularly characterized (Melo et al., 2016; Silva et al., 2017; Lima et al., 2019), indicating that there are a variety of genotypes of the protozoan in the insular environment.

Due to the absence of studies related to the epidemiology of *T. gondii* in the human population in the archipelago of Fernando de Noronha, the objective of this study was to determine the prevalence and risk factors associated with infection in individuals living on this island.

Material and Methods

Ethical aspects

The study strictly followed the ethical principles in research with humans, and was approved by the Research Ethics Committee of Universidade de Pernambuco (n. 19344819.4.0000.5207).

Study area

The study area was the Fernando de Noronha Archipelago (Figure 1), which comprises 21 islands and islets and has an area of 17,017 km^2 and an estimated population of 3,061 (IBGE, 2019). It has tropical climate comprising two seasons, one dry (August–January) and another rainy (February–July), with an average annual temperature of 26–27ºC (IBAMA, 1990).

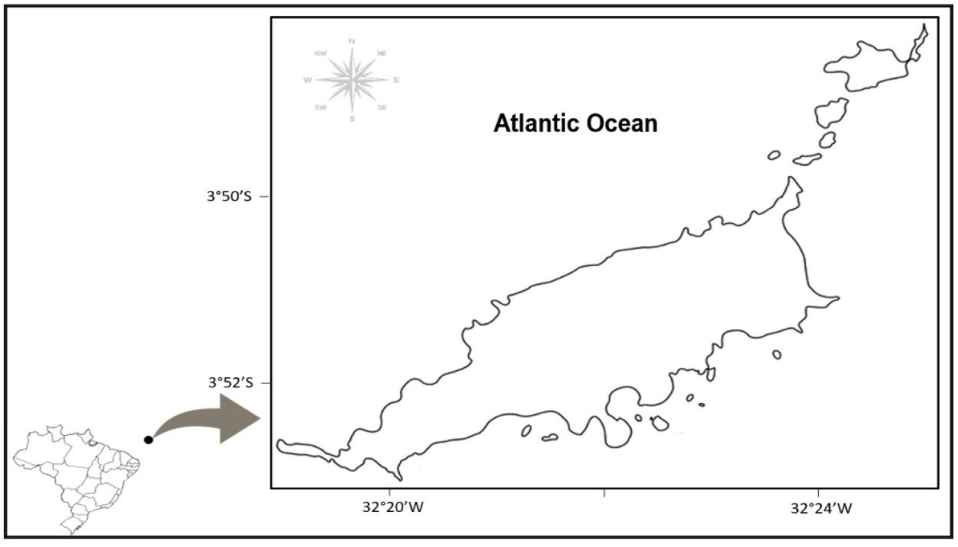

Figure 1. Map of the archipelago of Fernando de Noronha, Pernambuco, Brazil.

Sampling

This study was a cross-sectional epidemiological study. In order to determine the representative sample size, we applied the calculation for finite populations considering an estimated population of 3,061 individuals residing on the island of Fernando de Noronha in 2019, according to the Brazilian Institute of Geography and Statistics. Furthermore, an expected prevalence of *T. gondii* infection of 50% was assumed given the non-existence of previous data at a confidence interval (CI) of at least 95% with a statistical error of 5% (Thrusfield, 2007). This calculation resulted in a sample of 341 individuals.

The inclusion criteria for the study included both male and female individuals who were residents of the island for at least 6 months and were older than two years of age. Non-residents (tourists) of the island were excluded from the study. Participants were informed about the research objectives, and those who agreed to participate in the study received and signed a copy of the Informed Consent Form (ICF), according to Resolution No. 466/12 of the National Health Council (Brasil, 2012).

Collection of blood samples

Approximately 5 mL blood samples were harvested using clot separator gel tubes (BD Vacutainer®, USA) with disposable syringes and needles, the samples were kept at room temperature (25ºC) until the visible retraction of the clot. The samples were centrifuged at 1500 xg for 5 min, and serum aliquots were obtained and placed in microtubes, identified, and stored at -20ºC until further processing.

Serological analysis

Serum samples were evaluated by the indirect fluorescent antibody test (IFAT) to detect the presence of immunoglobulin G (IgG) anti-*T. gondii* antibodies according to the method proposed by Camargo (1964). A cut-off point of 16 was adopted (Dubey, 2010), and known positive and negative controls were included in all reactions. *T. gondii* tachyzoites from strain ME49 were used as antigens in slide preparation.

The sera were diluted in phosphate buffered solution (PBS, pH 7.2) at room temperature and were placed in slide wells and incubated at 37°C for 30 min in a moist chamber. The slides were washed three times for 10 min in PBS, followed by incubation (37ºC for 30 min in a moist chamber) with IgG monoclonal anti-human conjugated to fluorescein isothiocyanate (Sigma Chemical®, USA), diluted 1:100 in PBS containing 0.05% Evans Blue (Sigma Chemical®, USA). The slides were washed again, dried in an oven, and covered with buffered glycerol and coverslips. Finally, the slides were examined using an epifluorescence microscope (Axio Vert.A1 Zeiss, 40× objective) by double blind reading by two independent and previously trained individuals. If the results differed, a third individual performed a final reading.

Descriptive statistical analysis was used to calculate the absolute and relative frequencies of the results obtained in the serology.

Analysis of risk factors

Pre-structured questionnaires were applied to assess the risk factors associated with *T. gondii* infection comprising objective questions that addressed demographic information (age, address, and schooling) and the hygienic sanitary habits and dietary habits of individuals associated with the epidemiology of toxoplasmosis. The questionnaires were administered by staff trained for this purpose.

In order to identify the risk factors associated with *T. gondii* infection in humans on the island of Fernando de Noronha, a univariate analysis was initially conducted by Fisher's exact test, adopting a confidence interval [CI] of 95%. Variables with $p<0.25$ were used for reassessment in logistic regression models. Calculations were performed using Epi Info 7.2.3.0 (Centers for Disease Control and Prevention (CDC), with significance set at $p<0.05$.

Results

The prevalence of IgG anti-*T. gondii* antibodies in humans living on the island of Fernando de Noronha was 50.4% (172/341, 95% CI: 45.2%–55.7%). The sample studied was mostly composed of females 56.8% (194/341) aged 21–40 years 54,6% (106/194) and had studied up to high school 47,9% (93/194).

The univariate analysis of *T. gondii* infection in residents of the Island of Fernando de Noronha is shown in Table 1. The analysis of risk factors revealed that individuals who consumed well water or rainwater were about 2.43-fold more likely to be infected (p=0.020) compared to those who drank bottled or tap water. Individuals who consumed game meat had 1.80-fold increased likelihood of infection by *T. gondii* (p=0.026).

Table 1. Univariate analysis of infection by *T. gondii* in individuals residing on the island of Fernando de Noronha, Pernambuco, Brazil.

Variable	Univariate analysis		
	n	Positive (%)	p
Sex			
Male	147	74 (50.3%)	0.487
Female	194	98 (50.5%)	
Schools			
Elementary	73	50 (68.5%)	0.000
High school	166	89 (53.6%)	
University	92	26 (28.3%)	
Age range (yr)			
0-20	17	4 (23.5%)	0.000
21-40	174	70 (40.2%)	
41-60	123	80 (65.0%)	
> 60	17	11 (64.7%)	
Water Consumption			
Bottled	294	139(47.3%)	0.041
Tap	6	4 (66.7%)	
Well/Rain	35	24 (68.6%)	
Place where you have your meals			
Home	236	121 (51.3%)	0.245
Restaurants and Cafeterias	97	45 (46.4%)	
Disinfection of vegetables			
Water (only)	171	85 (49.7%)	0.582
Water + vinegar	58	32 (55.2%)	
Water + hypochlorite	105	49 (46.7%)	
How you eat meat			
Rare	64	34 (53.1%)	0.554
Well-done	180	93 (51.7%)	
Medium	83	37 (44.6%)	
Do not consume	6	2 (33.3%)	
Consumption of meat during cooking			
Yes	136	75 (55.2%)	0.067
No	199	92 (46.2%)	
Consumption of game			
Yes	77	47 (61.0%)	0.017
No	258	120 (46.5%)	
Presence of cats at home			
Yes	109	54 (49.5%)	0.515
No	226	113 (50.0%)	
Contact with cats			
Yes	141	64 (45.4%)	0.091
No	193	103 (53.4%)	
Practice gardening			
Yes	103	56 (54.4%)	0.162
No	232	111 (47.8%)	

Discussion

This is the first epidemiological study of *T. gondii* infection in humans on the island of Fernando de Noronha. The seroprevalence of antibodies found in human on this island (50.4%) differs from the prevalence rates found in other insular environments in different regions of the world: Islands of Jeju (ELISA: 13.2%) (Hong et al., 2011) and Gangwha-gun (ELISA: 25.8%) (Yang et al., 2012), both in South Korea, Island of Cebu, in the Philippines (Latex Agglutination Test - LAT: 26.4%) (Ybañez et al., 2019) Island of Sri Lanka (ELISA: 29.9%) (Iddawela et al., 2017), Island of Pulau Pangkor, Malaysia (ELISA: 59.7%) (Ahmad et al., 2014), Island of São Tomé and Príncipe, in West Africa (LAT: 63.1%) (Fan et al., 2012), Island of Granada (ELISA: 57%) (Asthana et al., 2006), and 10 island countries (Modified Agglutination Test - MAT: 39.8%) (Dubey et al., 2016), located in the Caribbean, San Carlos Island, Venezuela (Indirect Haemagglutination Assay- IHA, 49.8%) (Chacín-Bonilla et al., 2003).

Importantly, due to the wide dissemination of *T. gondii*, the seroprevalence in humans varies significantly across different regions of the world whether continental or insular. In Brazil, the prevalence of infection by this coccidium in humans is particularly high, and can reach more than 90% in some regions (Flegr et al., 2014). In addition to the environmental characteristics, cultural and biological factors also influence rates of T. *gondii* infection, and the serological test chosen is influenced by its sensitivity and specificity (Dubey et al., 2010; Morais et al., 2016). In an attempt to understand the reasons for the variations in the prevalence even in geographically close areas, it is important to investigate the risk factors associated with this infection.

The analysis of risk factors identified the consumption of well water or rainwater (OR=2.43) and consumption of game meat (OR=1.80) as being associated with increased likelihood of *T. gondii* infection.

The storage conditions of well water or rainwater are usually precarious and may be accessible to animals, for example cats that are able shed *T. gondii* oocysts into the environment. Taking into account the high density of cats on the island of Fernando de Noronha, the large number of oocysts eliminated in the feces of cats can be disseminated to storage tanks by rainwater and can even contaminate surface water and thereby serve as an important source of infection in the population, particularly when water intended for consumption is not filtered or boiled (Flegr et al., 2014; Dias et al., 2017; Robert-Gangneux & Dardé, 2012). This finding can be related to data from the Island of San Carlos in Venezuela, which raised the problem of islet infection from water contaminated by oocysts due to the close proximity of the cats to their homes, and consequently the water source of their inhabitants (Chacín-Bonilla et al., 2003). Oocysts are resistant forms of the parasite and can remain viable for up to 54 months in cold water (Dubey, 1998). In Brazil, outbreaks of toxoplasmosis and serological surveys have demonstrated the importance of untreated, filtered or boiled water as the main source of infection in humans, as noted in major outbreaks in Rio de Janeiro (Bahia-Oliveira et al., 2003), Paraná (De Moura et al., 2006), Pará (Carmo et al., 2010) and Rio Grande do Sul (Rio Grande do Sul, 2018).

Game hunting for subsistence is a common activity in island environments as a way to meet the protein needs of the population (Yang et al., 2012). Hunting and consumption of animals such as the rock cavy (*Kerodon rupestres*) and the tegu lizard (*Salvator merianae*) is common among the residents of the Island of Fernando de Noronha. A study carried out on the Island of Fernando de Noronha reported 58.3% positivity for anti-*T. gondii* antibodies in rock cavies (Lima et al., 2019). The high prevalence of antibodies in rock cavies indicates that the consumption of this animal could be a potential source of *T. gondii* infection among the inhabitants of the island. Previous studies conducted on the island of Fernando de Noronha showed high *T. gondii* seropositivity in livestock (Costa et al., 2012; Magalhães et al., 2016a,b; 2017). Although it does not represent the main source of meat in the island, these animals are a part of the diet and when infected by *T. gondii* can contribute to its transmission. Despite the fact that the ingestion of raw and/or rare meat was not identified as a significant risk factor for *T. gondii* infection in our study, it is important to highlight that there were higher frequencies of infection between individuals who consumed rare meat (53.1%) or those who the meat during preparation/cooking (55.2%).

With respect to gender, the study sample comprised mainly women (56.8%), the majority being of reproductive age, and 49.5% presenting negative serology for *T. gondii*. This suggests that there is a significant number of women susceptible to primary infection, which is worse if it occurs during the gestational period, as transplacental transmission can occur, which may pose a risk to the fetus (Walcher et al., 2016).

The univariate analysis showed a significant association between increasing age and seropositivity for *T. gondii*. This result corroborates with other studies, which found that increasing positivity is directly related to age, probably because older individuals have a greater chance of contact with the agent than do younger individuals (Ybañez et al., 2019; Chacín-Bonilla et al., 2003; Carmo et al., 2010; Gebremedhin et al., 2013; Aloise et al., 2018), but the rate of

infection with age can also vary according to the environment and socioeconomic level (Bahia-Oliveira et al., 2003; Mareze et al., 2019).

With respect to schooling, a higher prevalence of infection was observed in individuals with elementary schooling (68.5%). Previous studies have reported that the higher the schooling, the higher the information on preventive care for toxoplasmosis that reduces the risk of infection (Mareze et al., 2019; Aloise et al., 2018; Inagaki et al., 2014; Avelar et al., 2017, 2018), which reiterates the importance of education and health promotion to prevention of this disease.

It is important to note that prevalence studies are limited in establishing a true cause and effect relationship, since in these types of studies, there is generally no evidence of a temporal relationship because exposure and outcome are assessed simultaneously, without longitudinal data.

Conclusion

This is the first study to provide epidemiological information of *T. gondii* infection among the residents of the Island of Fernando de Noronha, revealing a considerable antibody seroprevalence in this population. The risk factors identified in this study suggest that the population should be advised not to consume wild animals from the island, and as well as promote the use of quality water. Appropriate treatment methods for well water or rainwater, for example, filtration or boil, should be a priority for health authorities.

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